UTTAR PRADESH JOURNAL OF ZOOLOGY

43(13): 75-79, 2022
ISSN: 0256-971X (P)

LIPOLYTIC ACTIVITY DURING METAMORPHOSIS OF *Maruca vitrata* (FABRICIUS)

S. A. JADHAV [aφ*] AND R. M. GEJAGE [b]

[a] Department of Zoology, Y. C. College of Science, Karad, District Satara – 415124, (M.S.), India.
[b] Department of Zoology, Smt. K. R. P. Kanya Mahavidyalaya, Islampur, Tal Walwa, Sangli – 415409, (M.S.), India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2022/v43i133088

Editor(s):
(1) Dr. Osama Anwer Saeed, University of Anbar, Iraq.
Reviewers:
(1) Adhira M Nayar, University of Kerala, India.
(2) Muhammad Zafar, Emerson University, Pakistan.
(3) José Oliveira Dantas, Instituto Federal de Sergipe, Brazil.

Received: 03 May 2022
Accepted: 07 July 2022
Published: 12 July 2022

Original Research Article

ABSTRACT

Lipolytic activity during metamorphosis of *Maruca vitrata* (Fabricius) has been attempted. The maximum activity was observed in 5th day old pupa of *M. vitrata.*Lipolytic activity gradually increased from 1 to 5 day and decreased from 6 to 8-day pupae of *M. vitrata*. The main sources of energy and structural component during histogenesis are lipid. The physiological role of lipase during metamorphosis of *M. vitrata* is discussed.

Keywords: Protein; insect pupa; *M. vitrata* (F.).

1. INTRODUCTION

India is major pulse producing country. Spotted pod borer is one of the major harmful insect pests occurs from flowering stage. Spotted pod borer is serious pest of green legumes [1]. Its damage is exhibited by weaving unopened buds and flowers. The larva damages the reproductive parts of flower leading to poorpod formation. In later growth period, it behaves as a pod borer and completes its larval and pupal development inside the pod. The damage due to insect-pests are much higher in pulses than in cereals. Among the insect pests pod borers have been identified as the major constraints in increasing the productivity of pigeon pea [2]. Many insects species almost dependant on lipids for metabolic needs. Primarily lipids are stored in fat body. Insect has ability to utilize lipids as substrate for reproduction, embryogenesis, metamorphosis and flight. Lipids are used as means of communication or regulation of many physiological processes [3]. Lipases comprise group of enzymes of widespread phenomenon of the plant and animal kingdom. Function of lipid is catalysing the hydrolysis of triacylglycerol,

[φ]Research Student;
**Corresponding author: Email: sushmaajadhav88@gmail.com;*

diacylglycerol, monoacylglycerol to glycerol's and fatty acids. Mostly lipass found in plasma, salivary gland muscles and fat bodies in insect bodies [4]. However, the information about the lipaseactivity during larval development of *M. vitrata* rather scanty. In the present work, an attempt hasbeenmadeto measure lipase activity during metamorphosis of*M. vitrata* which is main source of energy and structural component during histogenesis.

2. MATERIALS AND METHODS

Damaged cow pea pods were collected from local market and cow pea farms.The larvae from infested pods were collected and provided with fresh cow peapods and then placed in insect rearing cage at laboratory conditions of 12h photoperiod, temperature 28 ± 2^0C and relative humidity 78%. The insect rearing cage has dimension of 45 cm length, 45 cm width and 45 cm height. Cage is made of wireguaze on all sides except the top, which is made of glass. The cage has an access door at the front side to place and remove required material. A full-grown larva bore large exit holes, leave the pea pods and pupates underneath the pods.Larval developmental period was found to be 13 days. The pupae were collected immediately after pupation and were kept in glass jar having 15 cm in height and 7 cm in diameter covered with muslin cloth until the immergence of the adults. The pupation period of *M. vitrata was* noted as 8 days. Newly emerged male and female moths of *M. vitrata* were kept as 10 pairs in round glass jar. Female longevity is found to be 7 days. Egg incubation period was found to be 5 days. Adults were placed in rounded glass jar, having 12 cm height and 30 cm in diameter and covered with muslin cloth until the deposition of eggs. Adults were fed with10% sucrose solution. Pea pods were placed in rounded glass jar as an oviposition site and removed daily. A female moth lays 350 eggs. Life cycle is completed in 33 days. Rearing of *M. vitrata* was attempted according to method of Sharma [5].

Triacylglycerol lipase assay: Triacylglycerol lipase assay contains 0.25 L of substrate; 0.25 L partially purified pupal lipase enzyme and 1L of phosphate buffer (pH 7.7)in total volume of 1.5 L [6]. The incubation was carried out in glass stoppered conical flask for 25 minutes at 37^0C temperature in digital shaker. The reaction was stopped with 2 L of Cu-TEA reagents, then after15minutes 10 L of chloroform was added. The contents were shaken vigorously and kept for the separation of aqueous and organic phases. After 15 minutes upper phase was removed and 5 L of chloroform phase was transferred to centrifuge tube. Then 2 L of water was added without mixing and the tubes were centrifuge for few minutes. The upper water layer was removed carefully and exactly 2 L of chloroform phase was taken in another stoppered test tube. Then 1L of colour reagent containing diphenylcarbazone and diphenylcarbazide was added. At the end liberated fatty acids were measured calorimetrically. The absorbance was read at 540nm [7]. Triacylglycerol lipase activity from developmental stages of pupae was expressed in terms of specific activity as µmol of free fatty acids liberated / mg of protein /minutes.

The Lowry method for protein quantitation: The protein quantitation was attempted according to method of Lowry et al. [8]. Bovine serum albumin (100 mg /mL) was used as standard protein. The homogenates of pupae were prepared in chilled distilled water. The homogenate was prepared by using glass mortal pestle. Protein assay contains 0.5 L homogenate, 4.5 L of reagent I mixed well and allowed to stand for 10 minutes of incubation at room temperature. Immediately, 0.5 L reagent II was added rapidly performing the total volume of 5.5 L. After 30 minutes of incubation reading was taken calorimetrically at 750 nm. Reagent I and Reagent II were prepared freshly just before experiment.

Estimation of total lipid: The lipid estimation was attempted according to method of Bozdogan et al. [9]. Olive oil (1mg/mL) was used as standard lipid. 0.2 mL of 1 per cent pupal homogenate in test tube. Then added 1 mL mixture of chloroform and methanol and kept in water bath at 100^0C until complete evaporation. 0.18 mL Conc. $H_2SO_4$was added, vortex tubes and heated in water bath at 100^oC for 2 minutes. Immediately, 0.5 mL vanillin-phosphoric acid was added and incubated at 37^oC for 15 minutes. Then reaction mixture was cooled keeping in dark box for 45 minutes and purple colour appeared. Optical density was measured by colorimeter at 540 nm.

3. RESULTS AND DISCUSSION

3.1 Results

The metamorphosis was complete in 8 days. The maximum lipase activity was observed in 5-day old pupa. Lipolytic activity gradually increased from 1 to 5 day and decreased from 6 to 8-day pupae of *M. vitrata*. The K_m value of pupal lipase is 0.095×10^{-2} mM. Lipolytic activity during pupal development of *M. vitrata*is shown in Fig. 1.

3.2 Discussion

Effect of pH and temperature show that lipase activity optimum at 37°C and pH 7-9 and specific activity ranging from 0.52 to 0.82 [10]. Lipase activity in

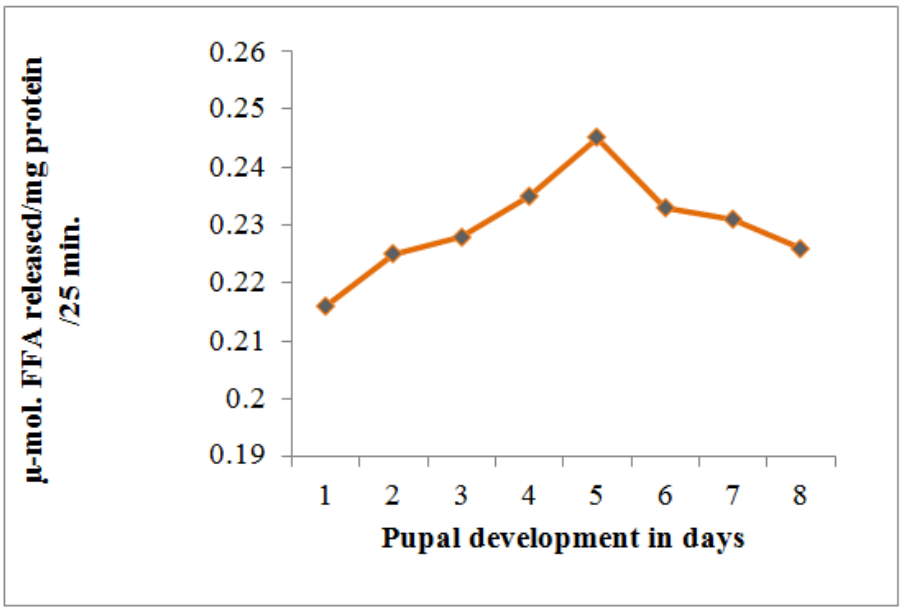

Fig. 1. Lipolytic activity during pupal development of *M.vitrata*

Table 1. Total protein, lipase activity and lipid in pupal stages of *M. vitrata*

Sr. No.	Pupal stages (Days)	Specific activity µmol FFA/mg Protein/25min.	Protein Amount (mg/mL)	Lipid Amount (µg/mL)
1	1	0.216	18	20.00
2	2	0.225	19	18.09
3	3	0.228	20	15.71
4	4	0.235	21	10.38
5	5	0.245	22	10.00
6	6	0.233	21	11.90
7	7	0.230	20	16.19
8	8	0.226	19	22.85

Helicoverpa armigera revealed at optimum pH 8.1, 20 min incubation time temperature 37°C and Km value 0.246 × 10^{-2} mM [11]. Lipase activity during metamorphosis from larva to pupa undergo dramatic changes in morphology and metabolic processes [12]. The maximum lipase activity gradually increases from 1to 5 day in *Earias vittella* [13]. Activity of purified lipase was revealed at pH 8 and highest activity was recorded between 37°C to 40°C [14]. Characterization of digestive enzymes from larval and adult midgut of *Papilo polytespolytes* revealed at 7.8 pH [15]. Optimal temperatures for soluble and membrane bound lipase noted as 35 and 50°C respectively [16]. Lipase activity noted at 7.7 pH, enzyme concentration 1% and Km value 0.129×10^{-2} observed in *H. armigera* [17]. Changes during pupal development maximum activity noted at pH 7, 30 min. incubation time and 5% substrate concentration [18]. Gejage and Gejage [19] have been noted lipase activity during larval development of an insect, *Leucinodes orbonalis* (Guenee). Pawar et al. [20] studied lipolytic activity during metamorphosis of *H. armigera*. Lipase activity during early pupal, mid pupal and late pupal stage is 5.5 µg/mg, 2.9 µg/mg, 3.2 µg/mg respectively [21]. Partial characterization of lipase revealed pH 8.2, incubation time 30 min., enzyme concentration 1%, temperature 37^0C and substrate concentration 5% in female adult of *L. orbonalis.* The maximum lipase activity was noted in 3day female adult of *L orbonalis* [22]. Lipase activity revealed highest at 6.0 pH in *Chilo partellus* [23]. Larval lipolytic activity revealed pH 7.9, incubation time 30 minutes, temperature 37^0C, enzyme concentration 1%, substrate concentration 5%in the larvae of *L.orbonalis.* Gradual increase in fat body lipase activity was observed from 6^{th}day larvae to 8^{th}day larvae and gradual fall from 8^{th}day larvae to 11^{th}day larvae of *L. orbonalis* [24]. In present study, the increase in lipolytic activity from 1-day to 5-day pupae indicates utilization of lipid for histolysis and role of lipase in histolysis which provides energy for metamorphosis and also materials for histogenesis of

M. vitrata. The specific activity of 1st day and 5th day pupae of *M. vitrata* was found to be 0.216 and 0.245 µg free fatty acids/ mg protein/ 25 minutes respectively. The decrease in lipase from 5-day to 8-day pupae suggest completion of histogenesis. The specific activity of 11th day pupa was found to be 0.226 µg free fatty acids/ mg protein/ 25 minutes respectively. The maximum lipase activity in 5-day pupae indicates more rate of histolysis and energy required for structural component mainly derived from lipid.

In present study, the pupal lipid content of 1st day and 5th day pupae of *M. vitrata* 20 and 10 µg/mL respectively indicate lipase activity is inversely proportional to the lipid content. One-way analysis of variance (ANOVA) between larval and pupal stages $p \leq 0.00$ and $F > 21.97$ indicates true hypothesis with significant differences ($p \leq 0.05$ and $F > 1$) in triacylglycerol lipase during larval and pupal developmental stages of *M. vitrata.*

4. CONCLUSION

The triacylglycerol lipase activity of 1-day pupae of *M. vitrata* was 1.13 fold less than 5-day pupae. The triacylglycerol lipase of 8-day pupae was 1.08-fold less than 5-day pupae. The increased levels of lipolytic activity in pupal stages may be due to higher rate of lipolysis to cope up with the high energy demand at pupal stages of growth and development.

ACKNOWLEDGEMENT

We are grateful to Principal, Dr. Vilas Kale, Smt. K. R. P. Kanya Mahavidyalaya, Islampur and Principal, Dr. S. B. Kengar, Y. C. College of Science, Karad for administrative help in research work. We are also thankful to SARTHI, Pune for providing JRF/SRF.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mahalakshmi MS, Sreekanth M, Adinarayana M, et al. Journal of Applied Zoological Research. 2002;13 (1):10-13.
2. Sahoo BK, Senapati B. Effect of pod characters on the incidence of pod borers in pigeon pea. Journal of Applied Zoological Research. 2002;13(1):10-3.
3. Gilbert LI, Chino H. Transport of lipids in insects. Journal of Lipid Research. 1974;15(5):439-56.
4. Pahoja VM, Sethar MA. A review of enzymatic properties of lipase in plants, animals and microorganisms. Journal of Applied Sciences. 2002;2(4):474-84.
5. Sharma HC. Bionomics, host plant resistance, and management of the legume pod borer, *Maruca vitrata*—a review. Crop Protection. 1998;17(5):373-86.
6. Hayase K, Tappel AL. Specificity and other properties of lysosomal lipase of rat liver. Journal of Biological Chemistry. 1970;245(1):169-75.
7. Itaya K. A more sensitive and stable colorimetric determination of free fatty acids in blood. J lipid Res. 1977;18(5):663-5.
8. Lowry OH, Rosebrough NJ, Farr AL, Randal J. Protein measurement with the folin-phenol reagent. J. Biol. Chem. 1951;193:265-275.
9. Bozdoğan H, Erbey M, Aksoy HA. Total amount of protein, lipid and carbohydrate of some adult species belong to curculionidae family (Coleoptera: Curculionidae). J. Entomol. Zool. Stud. 2016;4(5):242-8.
10. MsangoSoko K, Gandotra S, Bhattacharya R, Ramakrishnan B, Sharma K, Subramanian S. Screening and characterization of lipase producing bacteria isolated from the gut of a lepidopteran larvae, Samia ricini. Journal of Asia-Pacific Entomology. 2022;25(1): 101856.
11. Gejage RM, Pawar AS. Properties of lipase and lipolytic activity during ageing of female moth, helicoverpa armigera (Hubner). Indian Journal of Gerontology. 2021;35(1):1-7.
12. Peng J, Li Z, Yang Y, Wang P, Zhou X, Zhao T, Guo M, Meng M, Zhang T, Qian W, Xia Q. Comparative transcriptome analysis provides novel insight into morphologic and metabolic changes in the fat body during silkworm metamorphosis. International Journal of Molecular Sciences. 2018;19(11):3525.
13. Patil TC, Gejage RM, Bodare RD. Measurement of lipase activity during metamorphosis of Eariasvittella (Fabricius). Geobios. 2017;44:27-30.
14. Marepally L, Benarjee G. Isolation and purification of lipase from the midgut of fifth instar larvae of Antheraea mylitta drury. British Biotechnology Journal. 2016;12(4):1.
15. Gaikwad SM, Bhawane GP. Partial characterization of midgut enzymes in butterfly papilio *Polytes polytes* L.(Lepidoptera: papilionidae). 2015;10:43-53.
16. Delkash-Roudsari S, Zibaee A, AbbaciMozhdehi MR. Determination of lipase

activity in the larval midgut of Bacterocera oleae Gmelin (Diptera: Tephritidae). Invertebrate Survival Journal. 2014;11(1):66-72.
17. Pawar AS, Gejage RM, Dubal RS. Lipolytic activity during metamorphosis of *Helicoverpa armigera* (Hubner). Vivek Research. 2013;3(1):15-18.
18. Sakate PJ, Salunkhe PS. Study of lipase activity during development of *Chillo partellus*. Uttar Pradesh Journal of Zoology. 2013;33(1):61-68.
19. Gejage RM, Gejage MR. Lipase activity during ageing of Brinjal shoot and fruit borer *Leucinodes orbonalis* (Guenee). Indian Journal of Gerontology. 2010;24(4):413-420.
20. Pawar AS, Gejage RM, Dubal RS. Lipolytic activity in larval haemolymph of Helicoverpa armigera (Hubner). Uttar Pradesh Journal of Zoology. 2013;33(1):69-74.
21. Barsagade DD, Kelwadkar KM, Kadwey MN. Biochemical changes in the midgut during metamorphosis in Apr. s cerana indica. 2019;1(1).
22. Gejage RM, Gejage MR. Lipase activity during larval development of an insect, Leucinodes orbonalis (Guenee). Uttar Pradesh Journal of Zoology. 2010;30(3):293-6.
23. Pol JJ, Sakate PJ. Study on lipolytic activity profile during metamorphosis of Chilo partellus (Swinhoe). Uttar Pradesh Journal of Zoology. 2000;20(2):121-5.
24. Gejage RM, Manisha RA. Lipase activity in the fat body of developing larva of Leucinodes orbonalis (Guenee). Journal of Cell and Tissue Research. 2009;9(2): 1849.